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PERSISTENT BACULOVIRUS INFECTIONS

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I. SCOPE

This review will encompass persistent baculovirus infections. There are several excellent, recent reviews on persistent vertebrate viruses and virus diseases (1-9). The reader should refer to these reviews to obtain additional basic information on what is presently known about the nature of persistent virus infections. As well, this review does not address the persistence of baculoviruses in invertebrate hosts other than insects. The intent of this review is to draw together the information on baculovirus persistence in vivo and in vitro. We will also attempt to relate these findings to important findings in other virus systems in an attempt to present a probable scenario on baculovirus persistence and the role of persistence in baculovirus diseases in insects. Hopefully, the present review, which deals with evolutionarily simple hosts and immune systems, will facilitate a more general understanding of the mechanisms of virus persistence.

I. A. INTRODUCTION

The establishment of longterm infections in which the viruses, some of which are highly cytotoxic, continue to multiply while the host animal or cells continue to grow or divide are referred to as persistent infections. The classification of persistent infections utilized here is as outlined by Mims (6). According to this scheme there are three types or ways a virus can persist. First, the virus can persist without the shedding of infectious virus; second, infectious virus may be shed only periodically; and thirdly, virus replication and release occur continuously. Only the latter two types of persistence have been documented with baculoviruses. Other classification schemes have been put forth (9.0) and the readers

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are referred to them for additional information. The persistent infections of vertebrate viruses are modulated by many factors. Some of these are host factors such as cellular permissiveness, immune response including antiviral antibodies, and intracellular antiviral factors such as interferon. Other factors are virus derived. These include defective interfering particles, viral structural proteins, temperature sensitive mutants, etc.

B. PERSISTENCE OF VIRUS IN INSECTS

One feature of baculoviruses which has intrigued insect virologists and pathologists is their apparent ability to persist in an occult state in the host through many generations. This persistence is evident in the dramatic onset of virus epizootics in nature. The expression of infectious virus evidenced by the outbreak of acute disease in the population seems to be triggered by certain environmental conditions such as sudden temperature shifts, over population, or a change in diet (10,11). Although infection of larvae by ingestion of contaminating virus cannot be completely ruled out as the cause of these outbreaks, the fact that insect colonies which have not had any apparent contact with virus for several generations also exhibit these outbreaks, further implicates an occult or persistent virus as the cause. In addition, a number of researchers have reported the induction of baculovirus infections in insects by ingestion of a heterologous virus (12,13,14,15). All of these factors considered together strongly indicate that baculoviruses can persist in insects. It is not yet understood how these viruses persist in insects and what factors control the maintenance of the persistent state and the induction of infectious virus replication. Possibly the study of the persistence of these viruses in cell culture can provide clues to some of these questions.

C. BACULOVIRUS PERSISTENCE IN CELL CULTURE

There is currently only two systems in which the persistence of baculoviruses in cell culture have been studied in detail. The best studied is the non-occluded virus HZ-1 (Hz-1V). The persistence and molecular biology of this virus will consequently be predominant in this review. The other system is the persistence of the nuclearpolyhedrosis virus (SfNPV) of Spodoptera frugiperda in its homologous cell line IPLB-SF-21 (16).

A persistently infected IPLB-SF-21 cell line was generated by infecting cultures at a low multiplicity of infection (MOI) (4 TCID₅₀ units per cell). These cells reportedly showed typical signs of NPV infection, including polyhedral inclusion bodies (PIBs) about one week after infection. Cells in the population which appeared to be uninfected were subsequently subcultured. These cells (SFP) did not remain attached and continued to shed virus. Using an infectious center assay approximately 0.8% of the cells were found to be actively producing virus. Approximately 70% of the cells showed a positive reaction to fluorescently labeled SFNPV antibodies while only 3.0% of the cells contained PIB's. PIB's from SFP cells were found to be about 3 times less infectious for insects than PIB's produced by the standard SF virus in normal SF cells.

The best studied persistent baculovirus system is the HZ-1 virus system. This virus was identified as the persistent, adventitious agent in IMC-HZ-1 cells by Granados and co-workers in 1978 (17). The IMC-HZ-1 lines were originally isolated from adult ovarian tissue of Heliothis zea (18). These cells were found to be nonsusceptible to most insect viruses

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tested. This prompted Hink to suggest that they were infected with a persistent or latent virus (19). The first evidence of a persistent agent in these cells came from experiments in which the HZ-IV contained in these cells was induced into a productive state (20). In retrospect the attempts to transfect IMC-HZ-1 cells with DNA from Heliothis zea NPV, which resulted in CPE typical of an NPV infection without PIB formation (21) were most likely due to the induction of the persistent HZ-IV. The authors also reported that injection of the transfected IM-HZ-1 cells into H. zea larvae resulted in a typical NPV infection. This could be due to the presence in surviving IMC-Hz-1 cells of HZNPV DNA which was capable of infecting injected larvae. Vail et al. (20) also observed the induction of the persistent agent upon challenging IMC-Hz-1 cells with the heterologous virus ACNPV. The induction of HZ-1 virus in IMC-Hz-1 cells was confirmed by Kelley et al. (22). In this report it was determined that the homologous virus and heterologous viruses HZNPV, HaGV SfNPV, SiNPV as well as heat and UV inactivated TnNPV were all capable of inducing HZ-IV in IMC-Hz-1 cells. It was also reported that hydroxyurea, caffeine, bromodeoxyuridine, mitomycin C, and cycloheximide could not induce the virus. Iridescent virus type 22 also failed to induce the virus.

In addition to determining by electron microscopy that the baculovirus HZ-1 was the persistent agent in IMC-HZ-1 cells Granados et al. (17) found that the cell line established from Trichoplusia ni (Tn-368) was highly susceptible to the virus. Independently Ralston et al. (23) and Burand et al. (24) discovered that the virus isolated from IMC-HZ-1 cells could establish persistent infections in a number of lepidopteran cell lines including TN-368. Table 1 shows an example of cell lines tested in the

former study. Persistence in cell lines have been confirmed by serial passage, infectious center assays and DNA hybridization.

The finding that certain baculoviruses can replicate and persist in cultured cells has lead to more detailed studies of the factors involved in the persistence of the viruses.

D. CHARACTERISTICS OF CELL CULTURES PERSISTENTLY INFECTED WITH BACULOVIRUSES

Nuturing cells remaining after acute infections resulted in the establishment of cultures persistently infected with Hz-lV and SfNPV (16,23) Subcultures continually released infectious virus into the medium at lower levels. Hz-lV infected cultures, however, exhibited lytic infectious crises, generally before the 25th passage. During such episodes extensive CPE would develop and a concomitant rise in infectious titer of Hz-lV occurred. No such crises occurred with cultures persistently infected with SfNPV although there was a 100-fold variation in the level of infectious virus.

Cells in IPLB-SF-21 cultures persistently infected with SfNPV were smaller, grew slower and to a higher density than normal cells. They also did not remain attached to the culture vessel. The majority of TN-368 cells persistently infected with Hz-lV, in contrast, appeared normal in morphology and the density of such cultures were not obviously different.

Cells from cultures persistently infected with either of these viruses were resistant to superinfection by homologous virus but persistently infected SF cells were also somewhat resistant to Autographa californica NPV. Persistent cultures of TN-368 were as susceptible as the normal cultures to AcNPV. The homologous nature of the resistance to superinfection by Hz-lV

indicates a virus-specific mechanism while in the case of the SfNPV persistently infected cultures a more general antiviral factor, such as the suggested interferon-like molecules appears to be involved. The degree of DNA homology between HZ-lV, SfNPV and AcNPV are less than 1 percent (Smith and Summers, 1982).

E. ROLE OF DEFECTIVE PARTICLES IN HZ-1 VIRUS PERSISTENCE

Huang and Baltimore (3) have presciently outlined the role of defective interfering (DI) particles in viral diseases. The major characteristics of DI particles are that they are (1) composed of normal viral structural proteins, (2) contain only part of the virus genome and therefore reproduce only with the aid of a helper virus, and (3) interfere specifically with the replication of standard or parental virus. It is this last feature that plays an important role in the establishment and maintenance of persistent infections. Classically, in persistent virus diseases a balance of defective interfering and standard virus maintain diseases in a subacute or latent state until this balance is upset leading to overt symptoms of the disease.

In retrospect, indication that perhaps DI particles were involved in HZ-lV persistence came from the observed variability in the lengths of the virus particles (17,26). This heterogeneity was also reflected by the presence of minor molar bands in restriction enzyme digests of viral DNA (27,28). The presence of physically defective genomes in HZ-1 preparations was confirmed by contour length measurements of viral DNA (27). The molecular weight of HZ-1 DNA calculated from these measurements ranged from 75×10^6 to 158×10^6 . In these experiments the mode was 131 to 140×10^6 class.

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The first evidence of a biological role for DI particles in Hz-lV persistent infections was presented in the studies of Ralston et al. (23). Persistently infected cultures were shown to be resistant to superinfection by homologous standard Hz-lV but not heterologous AcNPV, a property characteristic of persistent infections modulated by DI particles (9). This feature indicates that the mechanism of persistence of Hz-lV is different from that of SfNPV discussed earlier.

Subsequently, Burand et al. (28) have shown a correlation between Hz-lV particle size, genomic heterogeneity and ability to establish persistent infections. They were able to isolate fairly homogeneous HZ-1 virus from a persistently infected TN-368 cell line by plaque purification. This virus (standard virus) would not establish persistent infections in TN-368. However, after ten serial high multiplicity passages of this virus, persistently infected TN-368 cells could be produced by the virus preparation. This ability to reestablish a persistent infection was accompanied by a 99% decrease in infectious virus titer and a rather dramatic increase in the level of physically defective particles. Electron microscope examination of these two virus preparations indicated that the mean particle length had decreased from 414 ± 30 nm for the standard virus to 370 ± 76 nm for the serial passaged isolate (Fig. 1). The large difference in standard deviations of these measurements reflects the heterogeneity in the virus population resulting from serial passage. This heterogeneity was also evident in restriction enzyme profiles of DNA purified from serial passaged virus. When compared with standard virus DNA, the Eco RI digest of serial passaged virus DNA indicated that a number of changes had occurred in the virus genome upon passage in cell culture (Fig. 2). These changes

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included an additional band, a number of insertions and deletions as well as several minor molar bands. Of particular interest are the minor molar bands H, P, and R which are also present in reduced amounts in nuclear extracts of persistently infected TN-368 cells. The decrease in the relative concentration of these bands not only documents the evolution of defective particles during the serial passage of HZ-1 virus but also indicates that defective particles are involved in the ability of the virus to persist in cell culture. This latter contention, however, remains to be verified experimentally.

The exact role of defective particles in HZ-1V persistence is not completely understood. Only through gaining a better understanding of the molecular events which occur during replication of the standard virus can we begin to understand the function of DI in events that occur during the establishing and maintenance of persistent infections.

F. OTHER FACTORS THAT INFLUENCE VIRUS PERSISTENCE

Cellular permissiveness influences virus multiplication through a variety of factors that interfere with virus replication and consequently affect the development and maintenance of persistent infections. The studies of Ralston et al. (23), besides providing evidence for the action of DI, also implicated cell type as a factor affecting the establishment and possibly the maintenance of HZ-1V persistent infections. Using cultures of TN-368 and two clones derived from it (29) it was demonstrated that persistently infected cultures could be established only in the parental TN-368 cells but not in cultures of two of its clones, Cl 10 and Cl 13 when replicate cultures were concurrently infected with a common inoculum.

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These results are consistent with the finding that persistent infections of vertebrate viruses are affected by cell type possibly through the generation and amplification of DI (30).

That cell culture populations contain a variety of cell phenotypes with different degrees of efficiency in supporting baculovirus replication was definitively shown with TN-368 and Autographa californica MNPV (29). More recently this cell line was shown to be semipermissive for SfMNPV (31) while earlier studies with the same virus and a clone of TN-368, CNL-1, suggested the cells were refractory to infection.

Interferon or other intracellular antiviral substances such as antiviral antibodies also interfere with virus replication resulting in abortive infections and DI particles (9). As noted earlier, the presence of interferon-like activity was suggested as a possible cause of resistance to superinfection of SfNPV persistently infected IPLB-SF-21 cells by both homologous and heterologous standard NPVs (16). No experimental biochemical evidence of such a factor was presented. Moreover, the recent successful construction by recombinant DNA techniques of an AcNPV expression vector and for production of human beta interferon in IPLB-SF-21 cells (32) argues against the action of similar proteins in the IPLB-SF-21-SfNPV system. However, the possibility remains that other virus-inhibitory cell products with different modes of action exist.

The immune system of insects has not been shown to function in a major role in baculovirus diseases. Because evidence for antibody-like activity in insect hemolymph is also lacking, if the immune system of insects affects baculovirus persistence, inducible enzymes such as lysozyme or polypeptides such as bactericidins and attacins that have been demonstrated

to function in the anti-bacterial immunological response of larval lepidoptera (33) are more likely candidates. To our knowledge, none of these substances have been tested for antiviral activity.

Virus derived factors other than defective interfering particles have been shown to interfere with standard vertebrate virus replication in insect cell cultures. Mosquito cell cultures that were persistently infected with the Togaviruses, Sindbis or Banzai, released into the culture medium proteins that interfered with replication of homologous standard virus (34,35). In the case of Banzai virus persistent infections serologic evidence indicated that the inhibitory protein is virus coat protein V-1. A recent study with vesicular stomatitis virus (36) in many cell types has suggested a possible mechanism of interference for virus coat protein. VSV transmembrane glycoprotein G synthesis results in the development of superinfection exclusion where the ability of the infected cells to take up virus is diminished.

It is possible that similar mechanisms operate in baculovirus persistence. However, evidence from preliminary experiments seeking low molecular weight substances soluble in medium of HZ-1V persistently infected TN-368 cell cultures that homologously interfere with standard virus replication have so far been negative (37).

G. REPLICATION OF HZ-1 VIRUS IN CELL CULTURE

The initial stages of the replication of HZ-1 virus in TN-368 cells observed by phase microscopy appear to be quite similar to other baculoviruses. Infected cells begin to round-up and by about 8 hours nuclear hypertrophy is observed. Unlike most other baculoviruses no PIBs are

observed and virus replication culminates in cell lysis with the release of the virus (Fig. 3). HZ-1V has typical baculovirus morphology (Fig. 1A). A close examination of the initial events of virus replication by electron microscopy revealed adsorption and penetration to occur by membrane fusion or viropexis as with the other baculoviruses. Uncoating of HZ-1V, like the granulosis viruses, occurs at the nuclear pore. Virus assembly in the nucleus of infected cells begins with the formation of membrane vesicles. The condensation of viral DNA and the assembly of the virus particle appears to occur inside this open vesicle. The vesicle is then completed yielding an apparently infectious enveloped nucleocapsid (Fig. 4A; 38).

The infectious virus particles move into the cytoplasm with the breakdown of the nuclear membrane. These particles then stack up at the cytoplasmic membrane but do not bud through it as do the NPVs. They are instead released upon cell lysis (Fig. 9B). In persistent HZ-1 infection only a few cells in the population lyse, while other cells maintain the virus in a persistent state. A key to understanding the persistence of this cytolytic virus is to determine how and in what state the persistent virus is maintained in the cells.

Employing a plaque-assay system the dynamics of HZ-1V replication in TN-368 cells was examined (Fig. 5). In these experiments both the level of infectious virus released from the cell, the extracellular virus (ECV), and the level of infectious virus remaining inside the cells, the cell associated virus (CAV), were measured. The CAV samples were collected from washed cells after brief sonication. These results show that the titer of CAV increases first starting at about 8 hr post infection (pi) followed by ECV at about 10 to 12 hr. The CAV titer peaks at about 15 to 16 hr pi while the ECV reaches a peak titer by about 20 hr.

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DNA replication in virus infected and control cells was followed by the incorporation of ^3H -thymidine into TCA precipitated fractions (Fig. 6). In virus infected cells there is a very curious drop in the rate of thymidine incorporation at 3 hr pi. This may represent the turning off of host cell DNA synthesis and the initiation of viral DNA synthesis. This drop is followed by a steady, dramatic increase in thymidine incorporation beginning at 4 to 5 hr and reaching a peak at 8 to 9 hr.

H. STRUCTURAL AND INTRACELLULAR POLYPEPTIDES

During the first 12 hours of virus replication 37 virus specific intracellular proteins were identified by pulse labeling with ^{35}S -methionine (Fig. 7). Using ^3H -mannose labeling, 8 of these proteins were found to be glycoproteins (Fig. 8). Based on the virus growth cycle, the pattern of DNA synthesis, and the appearance of these proteins (Table 2), the replication of HZ-1 virus was divided into three stages. These are: the early stage which include events occurring up to 4 hr pi; the intermediate stage including events occurring between 4 and 8 hrs pi; and the late stage which includes all virus specific events occurring after 8 hr pi.

Only one virus specific protein, the 75K protein, was detected during the early stage of viral replication. There is most likely other proteins made during this time period which are yet to be identified. The majority (29) of the virus specific proteins are first detected during the intermediate stage. During this period two types of virus regulation of protein synthesis are apparent. The first and most dramatic is the turning off of host cell protein synthesis early in the infection cycle of the virus. The second is the temporal control of viral protein synthesis evident in the

appearance and disappearance of specific viral proteins (72K, 75K, and 82K) during this period. Posttranscriptional modification of two virus proteins (109K, and 82K) was also detected during this period. It has recently been determined that most of the 109K protein, a highly glycosylated intracellular protein is deglycosylated giving rise to the 107K structural protein. The late stage is characterized by maturation of the virus which culminates in virion release through cell lysis. It is during this final stage that the virus structural proteins are made. Using 35(S)-methionine and the two sugars 3(H)-mannose and 3(H)-N-acetyl-glucosamine 28 virus structural proteins were identified (Fig. 9). Twenty six of these proteins labeled with 35(S) methionine and 14 with either one or both of the labeled sugars. Of these virus structural proteins only the 107K protein and two glycoproteins (72K and 75K) did not correspond to virus specific proteins detected at 12 hr pi. Because of the methods used and their level of sensitivity these 37 intracellular proteins and 28 structural proteins and glycoproteins most likely represent a minimum number of proteins coded for by HZ-1 virus genes.

I. POSSIBLE ROLE OF BACULOVIRUS PERSISTENCE IN INSECT VIRUS DISEASES

The persistence of baculoviruses within an insect population allows for the induction of the virus into productive infections resulting in diseased individuals, virus spread, and eventually an epizootic. During the later stages of a particular outbreak when virus spread and infections are at their peak defective virus particles could be generated along with standard virus. These defective particles are then capable of interfering with the complete replication of the standard or infectious virus. This would result in attenuated infections and possibly permit completion of the

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insect's life cycle. The emerging adults then would contain a self-limited infection and be capable of passing the virus on to future generations of insects. Such a mechanism involving DI particles incorporates the self-limiting viral disease process proposed by Huang and Baltimore (3) and extends its effect to the level of population.

J. SUMMARY

Details of the persistent infections caused by SfNPV and Hz-lV in their respective host cells, IPLB-SF-21 and TN-368 indicate that they are governed by different mechanisms. The characteristics of the two persistently infected cultures are different. Although both types release low levels of infectious virus the SFP cultures grow more slowly to a higher density, are smaller and do not remain attached to the culture vessel compared to normal cells while, in contrast, TN-368 cultures persistently infected with Hz-lV are close to normal in appearance. The resistance to superinfection by persistently infected cultures is only to homologous virus in the case of Hz-lV while that exhibited by SFP is more general in nature. The Hz-lV persistence evidence indicates that a factor of viral origin, defective interfering particles, function in modulating the infection. The SfNPV persistent infection is surmised from the data at hand, to be controlled by a more general anti-baculoviral factor(s), perhaps of cellular origin.

A proposed mechanism of how DI can function in insect diseases is presented. The role of DI in self-limiting disease has been extended to baculoviruses and to the population level to explain the subsidence of epizootics. More detailed molecular, cellular and virologic studies of

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persistent infections by baculoviruses are needed before additional generalizations about mechanisms can be made and the occurrence of processes hypothesized here tested.

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Fig. 1. Electron micrographs of phosphotungstic acid-stained (a) B1 and (b) B10 virus particles.

Fig. 2. EcoRI restriction enzyme digests of (a) B1 and (b) B10 virus DNA. The letters on the left indicate the individual fragments whose mol. wt. are given in Table 1. The asterisks indicate bands which are sub-molar, arrow indicates the position of an additional DNA fragment, large circle (O) indicates a fragment in which DNA has been deleted and the small circle (o) indicates fragments which contain additional DNA.

Fig. 3. HZ-1 virus-infected nucleus and appurtenant cytoplasm from a TN-368 cell. Note baculovirus morphology of HZ-1V and lytic nature of the infection.

Fig. 4. Assembly and maturation of HZ-1 virus in TN-368 cells. (A) Virus assembly in the nucleus of TN-368 cells commences with formation of membrane vesicles giving rise to enveloped nucleocapsids. Bar, 500 nm. (b) Virus particles released from the nucleus align near the cytoplasmic membrane and are eventually released by cell lysis. Bar, 1000 nm.

Fig. 5. Growth curve of HZ-1-B1 virus in TN-368 cells. Cells were infected at an MOI of 20 PFU/cell, and the levels of infectious CAV (O) and ECV(O) from 0 to 36 h p.i. were measured by plaque assay.

Fig. 6. Incorporation of [^3H]-thymidine in HZ-1 virus (o o) and mock-infected (o o) TN-368 cells. Cells were infected at an MOI of 20 PFU per cell and the level of radioactivity in TCA precipitable fractions was measured from 0-15 h p.i.

Fig. 7. Autoradiogram of intracellular polypeptides of control (C) and HZ-1 virus (V) pulse-labeled for 1 h with [^{35}S]methionine. TN-368 cells were infected at an MOI of 20 PFU/cell and starved for 1 h before the

time p.i., samples were collected and analyzed by PAGE. The apparent molecular weights (10^3) of the virus-specific intracellular polypeptides are shown at the right.

Fig. 8. Autoradiogram of intracellular glycoproteins of control (C) and Hz-1 virus (V) infected cells pulse labeled for 1 hr with 3-H-mannose. TN-368 cells were infected at an MOI of 20 PFU per cell and starved for 1 hr prior to labeling. After labeling for 1 hr during the indicated time, samples were collected and analysed by PAGE. The values on the right are the apparent molecular weights of virus specific intracellular glycoproteins $\times 10^{-3}$ daltons.

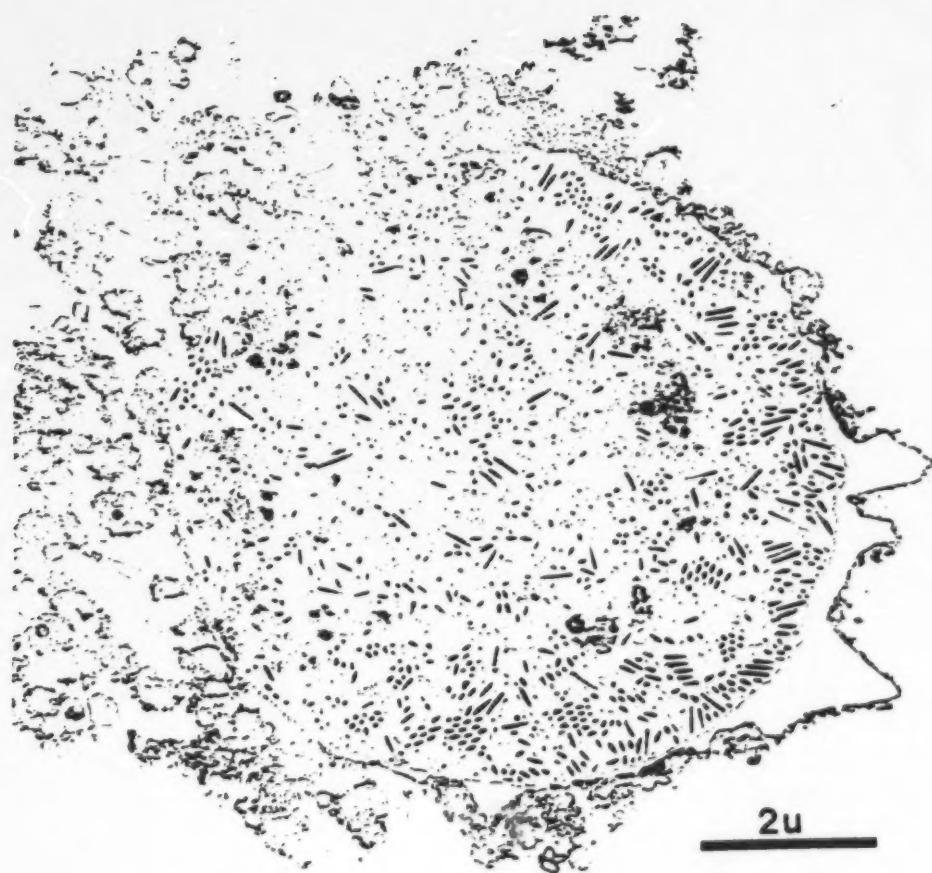
Fig. 9. Hz-1 virus structural proteins and glycoproteins. (A) Molecular weight standards; (B) [^{35}S]methionine-labeled viral proteins; (C) N-[^3H]acetylglucosamine- and (E) [^3H]mannose-labeled viral glycoproteins analyzed by PAGE. Lane D, Hz-1 virus labeled with N-[^3H]acetylglucosamine in the presence of 10 μg of TM per ml. In this lane, the virus particle concentration was approximately equal to that in lane C. Arrows, Virus proteins and their apparent molecular weights (10^3).

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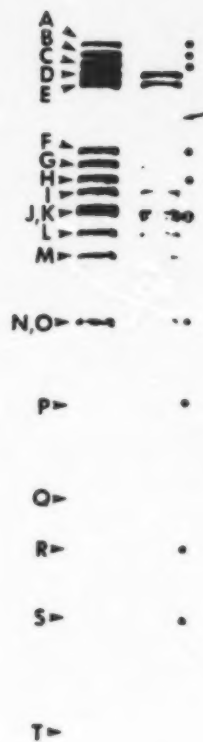
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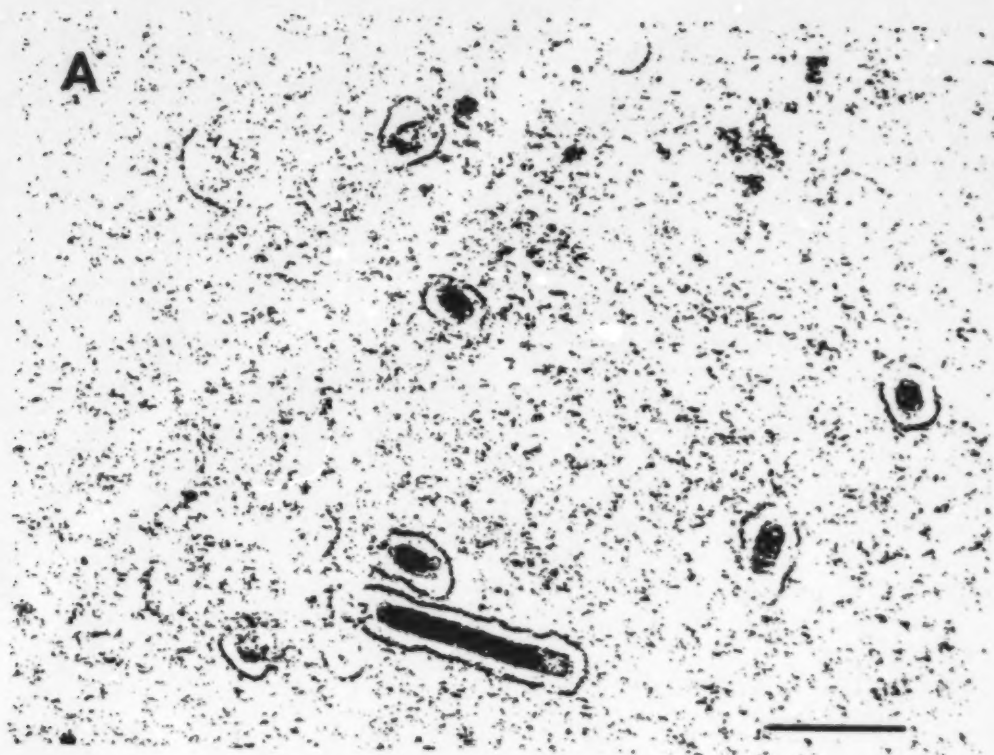
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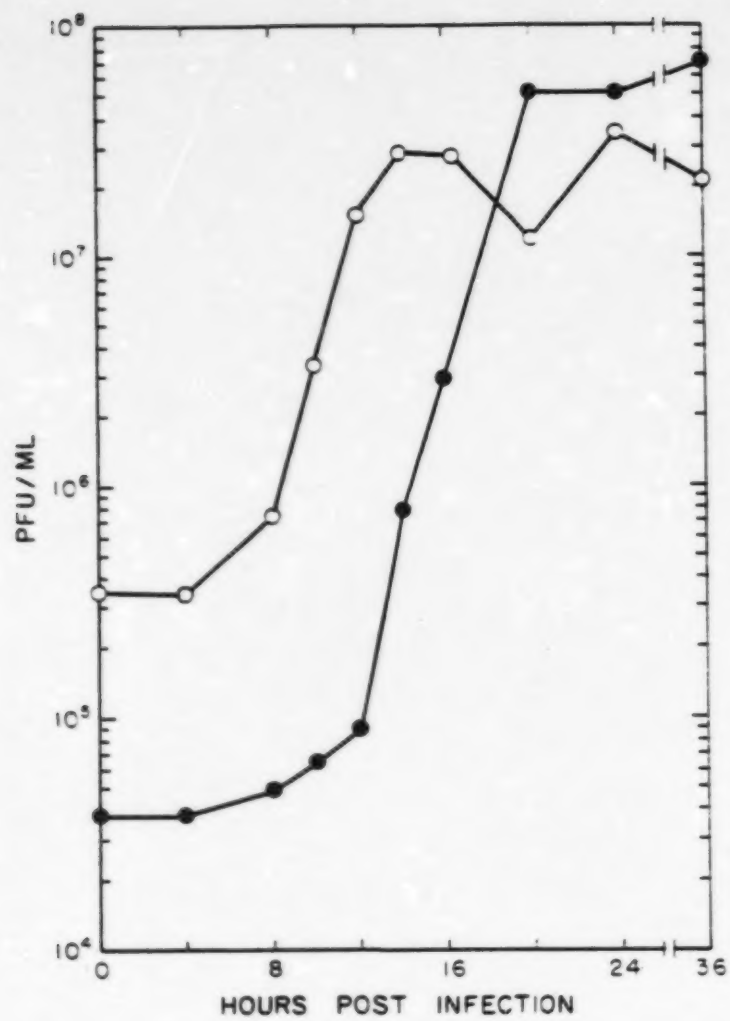
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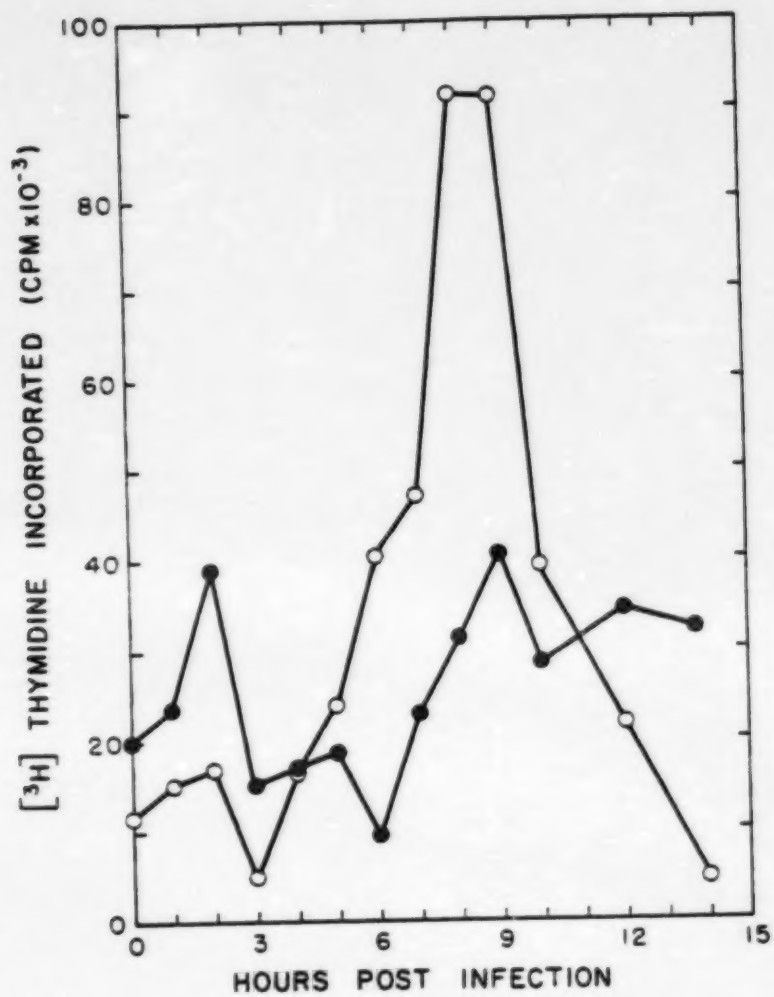
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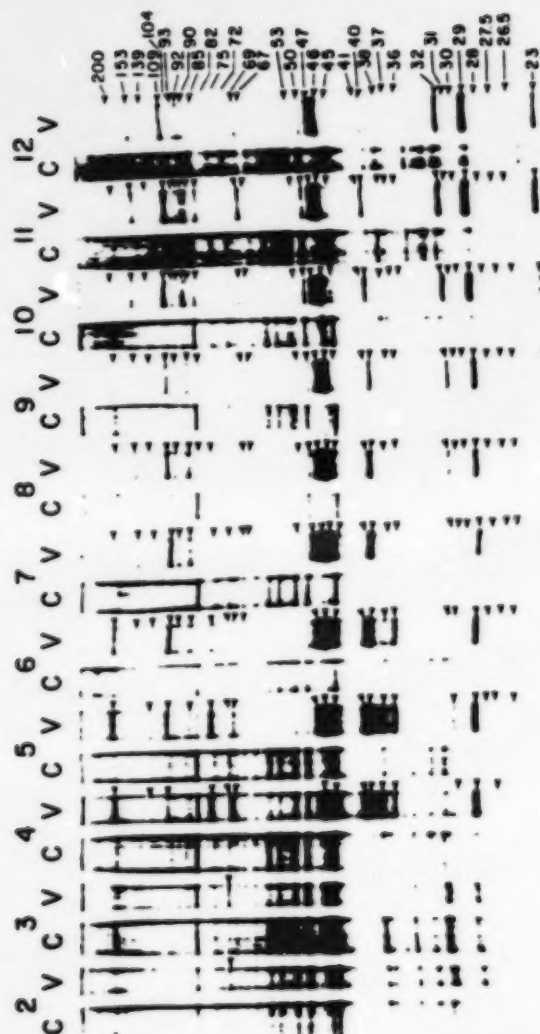


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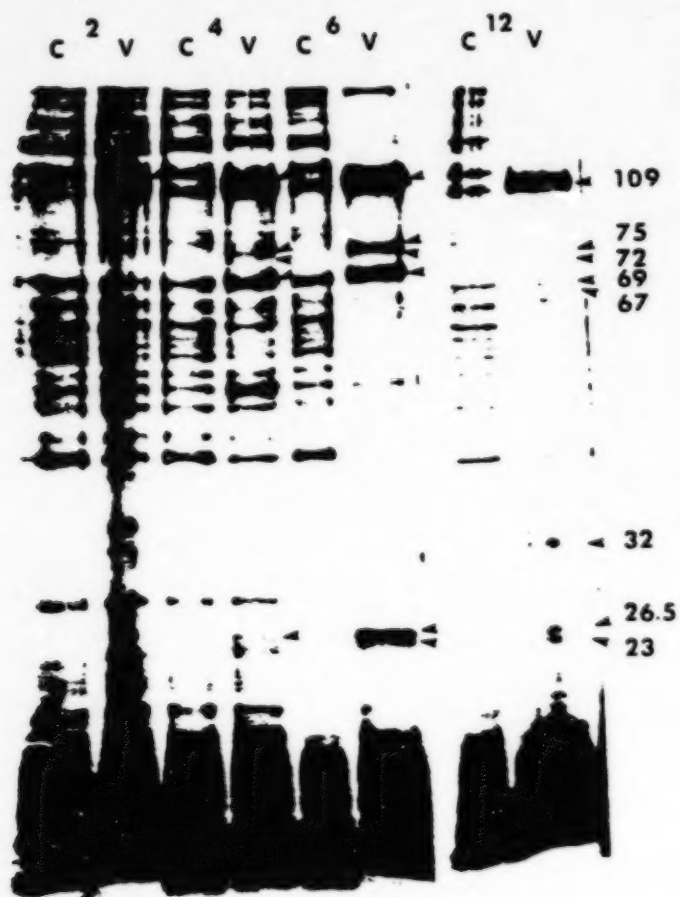








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